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Research Article

BIOLOGICAL ACTIVITY OF ESSENTIAL OILS OF TWO VARIANT OF *CINNAMOMUM VERUM* PRESL. FROM NORTH EAST INDIA ON *CALLOSOBRUCHUS CHINENSIS* (L.)

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ABSTRACT: *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) is one of the major insect pest infesting stored pulses in India. Recourse of synthetic insecticides to protect stored pulses often lead to dangers of the development of resistant strains, toxic residue and users safety. Locally available and less toxic pest management alternatives such as the use of effective botanicals are important. Essential oils of two variants of *Cinnamomum verum* Presl. Syn. *C. zelanicum* Blume from North East India (RRL J 1620 and RRL J 1622) were characterized and tested for their biological activity in vapour form against *Callosobruchus chinensis* (L.). Choice and no-choice tests showed that leaf and bark oil of two variants of *C. verum* Presl. had significant repellent action and reduced the fecundity and decreased egg hatchability of *C. chinenisis* (L.). However, leaf oil with higher percentage of eugenol exhibited better activity than the bark oil in both the tests. Hence, the volatile essential oils of both the variants of *C. verum* Presl. can be used safely as fumigants.
Key words: Biological activity, Essential oils, *Cinnamomum verum*, *Callosobruchus chinensis*

INTRODUCTION

The pulse beetle *Callosobruchus chinensis* (L.) is a major storage pest of pulses throughout India including its North Eastern part. In order to protect pulse grains in storage, we often have recourse to synthetic insecticides with their usual dangers of the development of resistant strains, high cost of application, toxic residues and users' safety. To avoid such undesirable consequences, many workers have focused their attention on the use of less hazardous practices and/or substances to protect stored products. Among the best different substances tested against insects attacking stored products are mineral oils, vegetable oils, essential oils, powders of dried citrus or grape fruit peels etc. (Su *et al*, 1972; Singh *et al*, 1978; Pereira, 1983; Sighamony *et.al*, 1986, Mishra, 2000; Stamopaulos, 1991).

Although, the mode of action of the plant material is not well known, it is generally believed that they provoke the death of insect eggs and larvae by suffocation or through fumigation effect (Schoonhoven, 1978), or in some cases, through antifeedant and repellent activity (Don-pedro, 1985; Alford *et al*, 1987). In most of the studies, the above substances have been applied to the surface of stored grains or mixed with them in different concentrations. However, it is possible that substance considered as 'innocuous' because of their origin, could still be hazardous to consumer when added to food.

Cinnamomum verum Presl. syn. *C. zeylanicum* Blume (Lauracae) is a small evergreen aromatic plant growing wild in the southern coastal regions of India up to an altitude of 2800 m and Cylon (Anon. 1950). In north eastern part of India, it is being grown in the homestead gardens by a large section of people to meet their domestic requirements of its spicy bark (Nath *et al*, 1997). It is the source of cinnamon bark, one of the major tree spices of greater commercial demand besides being useful in preparation of a number of products in traditional system of medicine (Thakur *et al*, 1989). The plant also yields two types of commercial essential oils. Its bark oil, rich in Cinnamaldehyde is mainly used in flavouring and perfumery industries, while leaf oil containing Eugenol as a major component is used mainly in seasoning and snacks (Pillai, 1993).

The present study was made to test the possible repellent effects of the vapours of the essential oils of two variants of *Cinnamomum verum* Presl. (RRL J 1620 and RRL J 1622) on *Callosobruchus chinensis* in laboratory condition.

MATERIALS AND METHODS

Plant material and isolation of essential oils

Leaves and bark of the two varieties (RRL J 1620 and RRL J 1622) were collected from individual trees of about 9-10 years old. Fresh leaves and fresh & ground stem barks of the plants were hydro-distilled separately using Clavenger type apparatus for 3 hour and 5 hour respectively. The essential oil thus obtained were dehydrated over anhydrous sodium sulphate and subjected to biological testing.

Analysis of essential oils

Analysis of the oils for their components were performed using a 10% OV-101 (2m x 2mm s.s.) column coated on chromosorb W-HP 80/100 mesh in a CIC-GC equipped with FID. N_2 was used as carrier gas at 25ml/min. Column temperature was performed from 100^0C-220^0C at 2^0C/min. where recorded chart speed was 0.25 cm/min. Identification of the components were done by comparing their relative retention times with those of the authentic compounds.

Testing of the biological activity

The adults of *C. chinensis* (L.) were obtained from laboratory culture maintained on seeds of blackgram stored in an indigenous bamboo storage structure in room condition.

Two-choice and no-choice bioassay was carried out to assess repellent activity. The apparatus used in the two-choice situation is prepared in the laboratory as described by Stamopoulos (1991). One 3 day old inseminated female beetle was placed within the arena, whilst in each of the 'satellites' ten blackgram seeds were used in order to induce oviposition. In the middle of the inside surface of each 'satellite' cover a small piece of synthetic sponge was glued to serve as vapour diffuser. About 20 small holes were also made in each cover using a No.000 entomological pin to ensure good aeration and humidity equilibrium and to avoid high concentration of oil vapour inside the apparatus.

In the no-choice test, one female beetle was placed with ten blackgram seeds in the same plastic vials as the 'satellites' of the two-choice test.

In each treatment 50µl of essential oil were applied to each diffuser while the control sponge was left untreated.

All the experiments were carried in the entomological laboratory of CSIR-NEIST, Jorhat in room temperature and humidity. In both the tests, the numbers of eggs laid and hatchability were recorded. The statistical analysis of the data was performed using Completely Randomized Block Design to evaluate performance of the treatment.

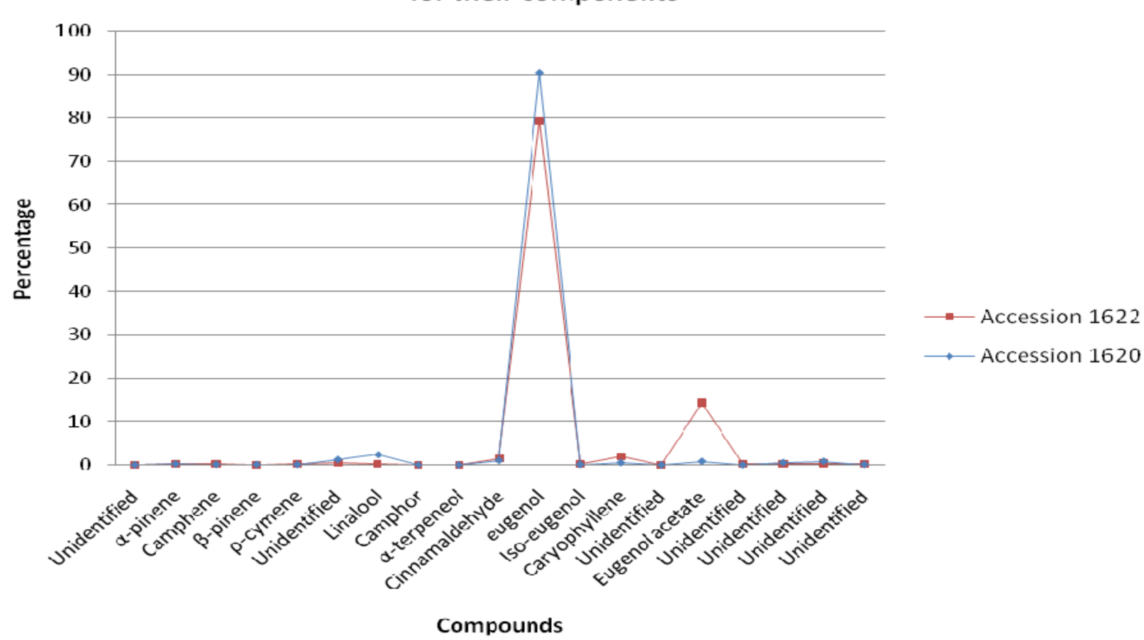
RESULTS AND DISCUSSION

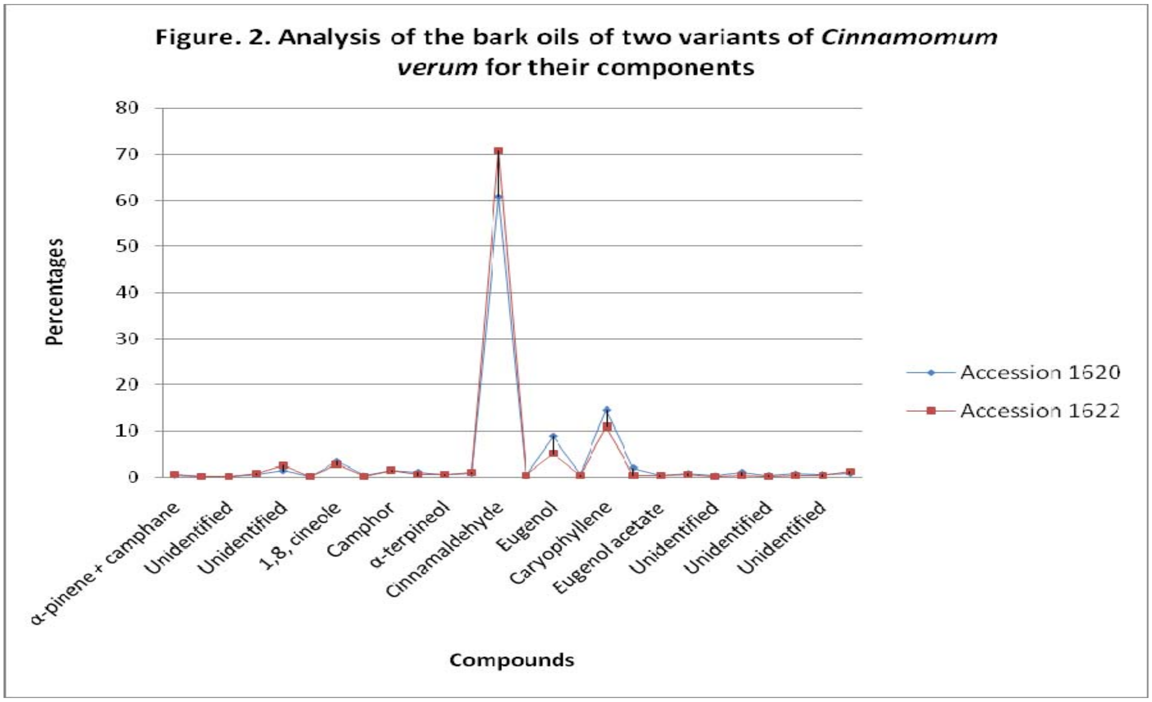
Analysis of oil

Analysis of leaf oils of two variants (RRL J 1620 and RRL J 1622) of *Cinnamomum verum* Presl. showed that major compound found in leaf oil is euginol (90.4% in Accession 1620 & 79.3% in Accession 1622). However, in Accession 1622, euginol acetate is more (14.3%) than in Accession 1620 (0.9%), as shown in the Figure 1.

The major component of bark oils of two variants (RRL J 1620 and RRL J 1622) of *Cinnamomum verum* Presl. is Cinnamaldehyde (60.7 & 70.9 per cent, respectively). Compared to leaf oils, the bark oil contains more Caryophyllene which is recorded as 14.5% & 10.9% in Accession 1620 & 1622, respectively, which can be observed in Figure 2.

Figure. 1. Analysis of the leaf oils of two variants of *Cinnamomum verum* for their components





Biological activity

The number of eggs laid and eggs hatchability in the two-choice test is give in Table 1. It is evident from the table that the presence of essential oil vapours of the two variants of *C. verum* appears to deter the females from laying their eggs on the treated seeds. There is a significant difference (P=0.01) between the control and oil treatment in both the case of ovipositional preference and hatchability. It is observed that all the oils tested act not only as oviposition deterrent but also adversely influence fecundity. However, the essential oils obtained from the leaves of the two variants are biologically more active than the oils extracted from the barks. Likewise, the essential oils of variant I (RRL J 1620) obtained both from leaves and barks showed more activity than the variant-II (RRL J 1622) of *C. verum.*

Table 1. Ovipositional preference and egg hatchability of *C. chinensis* in the presence of essential oil in two-choice test.

Treatment	Total eggs laid (%)	Egg hatchability (% of egg laid)
Control	187.67 (82.31)^{c}	166.00 (83.56)^{b}
Leaves oil (Variant-I)	40.33 (17.69)^{e}	32.67 (16.44)^{d}
Control	187.00 (81.19)^{c}	165.00 (82.78)^{b}
Leaves of (Variant-II)	43.33 (18.81)^{c}	34.33 (17.22)^{d}
Control	209.67 (77.94)^{a}	185.33 (81.05)^{a}
Barks oil (Variant-I)	59.33 (22.06)^{d}	43.33 (17.22)^{c}
Control	197.33 (76.39)^{b}	178.33 (18.95)^{a}
Barks oil (Variant-II)	61.33 (23.74)^{d}	47.67 (4.09)^{c}
S.E.M. (±)	2.95	2.92
C.D. (P=0.01)	8.61	8.51

In case of no-choice test also, the results (Table 2) showed that there is significant reduction in oviposition & hatchability of *C. chinensis* due to the presence of essential oil vapours. However, in this test, leaf oil of variant-II and barks oil of variant-I exhibited statistically indifference results in case of fecundity, whereas, egg hatchability in the above treatment was found to be significantly different.

The major percentage of major compounds differs in both the variants and in respect to the plant parts taken for oil extraction. Variant-I (RRL J 1620) contains comparatively higher amount of Eugenol in leaf (90.4%) and lower amount Cinnamaldehyde (60.7%) in stem bark oil and variant-II (RRL J 1622) contains comparatively lower amount of Eugenol in leaf (79.3%) and higher amount of Cinnamaldehyde (70.9%) in stem bark oil.

Table 2. Ovipositional preference and egg hatchability of *C. chinensis* in the presence of essential oil in no-choice test.

Treatment	Total eggs laid (%)	Egg hatchability (% of egg laid)
Control	218.67 (36.92)[a]	195.67 (89.48)[a]
Leaves oil (Variant-I)	78.67 (13.27)[d]	65.33 (83.04)[d]
Leaves of (Variant-II)	87.33 (14.74)[c]	71.00 (81.30)[d]
Barks oil (Variant-I)	94.33 (15.93)[c]	84.00 (89.05)[c]
Barks oil (Variant-II)	113.33 (19.13)[b]	95.00 (83.83)[b]
S.E.M. (±)	2.65	1.53
C.D. (P=0.01)	8.40	4.86

Out of the four oils screened leaf oil with higher percentage of Eugenol (90.4% & 79.3% in variant-I and Variant-II, respectively) showed better repellent against *c. chinensis* in vapour form which does not necessitate a seed surface application. The present findings have been supported by the findings of Stamopoulos (1991), Babu *et al* (1989) and Pathak *et al* (1997) who observed reduced fecundity, decreased egg hatchability and increased neonate larval mortality of bean bruchid. Therefore, it seems to be the most promising for possible safe use against this insect pest. The mode of action of these substances is not yet known and therefore, a further study seems to be necessary to ascertain its involvement in the physiology of oviposition of this important insect pest of stored pulses.

CONCLUSION

The vapour forms of essential oils act as oviposition deterrents and found to have adverse effect on fecundity and hatching of eggs of *Callosobruchus chinensis*. The two variants, (RRL J 1620 and RRL J 1622) of *Cinnamomum verum* Presl. were found to have very good effect in reduction of fecundity and egg hatchability of *C. chinensis.* However, the essential oils obtained from the leaves of the two variants where eugenol content is higher are biologically more active than the oils extracted from the barks.

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